9. U. Bors, C. Michel, and U. Saran, Biochim. Biophys. Acta, 540, 162 (1978).
10. K. S. Chio and A. L. Tappel, Biochemistry (Washington), 8, 2827 (1969).
11. J. R. Desiderato, J. Comp. Physiol. Psychol., 87, 208 (1974).
12. J. Folch, M. Lee, and G. H. S. Stanley, J. Biol. Chem., 226, 497 (1957).
13. S. L. Taylor, M. P. Lamden, and A. L. Tappel, Am. J. Clin. Nutr., 11, 530 (1976).
14. A. A. Shvedova, A. S. Sidorov, K. N. Novikov, et al., Vision Res., 19, 49 (1978).

HUMAN MUSCLE GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE IN ATHEROSCLEROSIS

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KEY WORDS: human muscle glyceraldehyde phosphate dehydrogenase; isolation; properties; atherosclerosis.

Changes in the functional activity and certain properties of glyceraldehyde phosphatase dehydrogenase (GAPD) from the skeletal muscles of rabbits with experimental atherosclerosis were discovered previously [2, 3].

The object of the present investigation was to develop a method of obtaining the enzyme in a pure crystalline form from the muscles of patients with atherosclerosis and to study the catalytic activity and some physicochemical properties of this enzyme.

EXPERIMENTAL METHOD

GAPD was isolated from autopsy material. Muscles were taken from 40 cadavers not more than 12-18 h after death. Death was accidental in all cases due to automobile and railroad accidents and gunshot and knife wounds. Persons dying from organic disease of the liver, kidneys, endocrine system, etc., were excluded. Material taken from subjects with no morphological signs of atherosclerosis was included in the control group.

GAPD was isolated in the crystalline form by preliminary extraction of the muscles with potassium phosphate followed by fractional precipitation with ammonium sulfate to remove ballast proteins. The method of fractionation of muscle proteins from human muscles in [1] was used as the prototype for development of the method in the present case.

EXPERIMENTAL RESULTS

Distinguishing features of GAPD in atherosclerosis came to light even at the stage of isolation of the enzyme. They were expressed, in particular as changes in the crystallization process: the earlier appearance of crystals but, at the same time, their low stability in the mother liquor, their smaller size, and a tendency to be arranged in groups. A typical feature of the enzyme from normal human muscle tissue is that it forms rod-shaped crystals with clear edges. The shapes of the GAPD crystals obtained by the present writers agree with those described in the literature [4]. The homogeneity of the preparations was confirmed by electrophoresis in polyacrylamide gel (Fig. 1). The GAPD was concentrated in the gel as a single band. Enzymes from muscles from patients with atherosclerosis migrate under similar conditions more slowly and are located nearer to the starting line.

Comparison of the specific activity of the enzymes from normal and atherosclerotic subjects showed that in the latter GAPD activity was 42% lower and amounted on average to 2.7 ± 0.2 μ-moles NADH/mg·min. GAPD from muscles of patients with atherosclerosis, when dissolved in wa-

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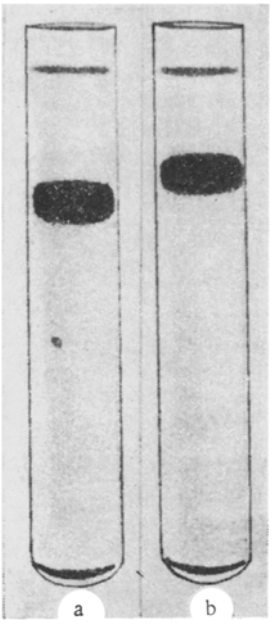

Fig. 1. Electrophoresis of GAPD from human muscles in polyacrylamide gel (7.5% gel in Tris-glycine buffer, pH 8.6). a) Normal; b) atherosclerosis.

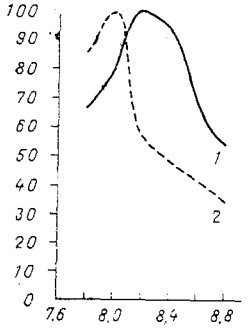

Fig. 2

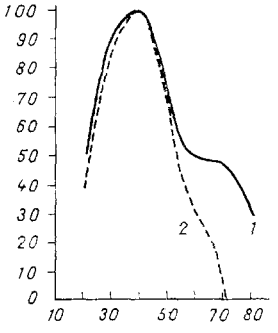

Fig. 3

Fig. 2. GAPD activity as a function of pH for normal (1) and atherosclerotic subjects (2). Abscissa, pH values; ordinate, GAPD activity (in %).

Fig. 3. Effect of temperature on activity of GAPD from muscles of normal subjects (1) and patients with atherosclerosis (2). Abscissa, temperature (in °C); ordinate, activity (in %).

ter without ammonium sulfate at 0-4°C, was unstable: On the 2nd day of storage, floccules and sedimentation began to appear. Under normal conditions the appearance of a sediment of the enzyme was observed on the 4th-5th day. There was no significant difference between the adsorption spectrum of the enzyme from normal and atherosclerotic subjects.

Investigation of GAPD activity as a function of temperature showed greater thermolability in atherosclerosis: The enzyme was completely inactivated at 70°C, whereas under normal conditions this occurred at a higher temperature. Maximal GAPD activity was found in both cases between 30 and 50°C (Fig. 2).

A comparative study of GAPD activity in medium with different pH values showed (Fig. 3) that optimal activity of the enzyme from normal human muscles occurred between pH values of 8.2 and 8.4 (0.08 M Tris-HCl buffer). In atherosclerosis the pH optimum was shifted to the acid side (pH 7.8-8.0).

The experiments thus demonstrated a decrease in the functional activity and changes in the physicochemical properties of GAPD, a very important enzyme of carbohydrate metabolism, in atherosclerosis. The possibility of obtaining crystalline enzymes from autopsy material allows the pathochemical basis of diseases to be studied at the molecular level.

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LITERATURE CITED

1. M. F. Gulyi, B. D. Dvornikova, A. M. Kolomiichenko, et al., in: Problems in Muscle Biochemistry [in Russian], Kiev (1954), p. 244.
2. L. T. Litvinenko, M. F. Gulyi, F. N. Gil'miyarova, et al., Ukr. Biokhim. Zh., No. 2, 214 (1972).
3. I. V. Sidorenkov and F. N. Gil'miyarova, Vopr. Med. Khim., No. 3, 250 (1970).
4. T. Baranowsky and M. Wolhy, Acta Biol. Med. Ger., 11, 651 (1963).

COMPARATIVE STUDY OF GAS-TRANSPORT CHARACTERISTICS OF EXTRAERYTHROCYTIC OXYGEN CARRIER MODELS

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KEY WORDS: hemoglobin; artificial oxygen carrier; gas transport characteristics.

During the attempt to create an artificial substitute for the erythrocytes of the blood, which has made rapid progress during the last decade, high-molecular-weight derivatives of hemoglobin (Hb) capable of circulating for a long time in the blood stream have been created [1, 6]. One of the main disadvantages of these compounds has been the lower efficiency of oxygen delivery compared with blood, which is also a feature of the original solutions of purified Hb and is due to the loss of 2,3-diphosphoglyceric acid (2,3-DPG) in the process of Hb isolation [7]. This problem was subsequently solved by the covalent addition of pyridoxal-5'-phosphate (PP), a functional analog of 2,3-DPG and a regulator of the affinity of Hb for oxygen, to Hb and its derivatives, so that the affinity of these Hb derivatives for oxygen is brought closer to the characteristic values for human blood [2]. A study of the principal physicochemical and biochemical properties of an Hb polymer (PHb) containing PP (PHb-PP) gave positive results [2] and laid the foundations for an extensive study of the gas-transport properties of the resulting compound.

The object of the investigation described below was to study the oxygen-dissociation curves (ODC) of PHb-PP and Hb-PP, the time course of their binding with oxygen at different Hb concentrations, and also the character of interaction with allosteric effectors under conditions as close to physiological as possible, a matter of the greatest importance when assessing the acceptability of these Hb derivatives as artificial oxygen carriers.

EXPERIMENTAL METHOD

ODC of the test compounds were recorded under physiological conditions (pH 7.4, pCO_2 40 mm Hg, temperature 37°C, Cl^- concentration 0.15 M) on a Blood Gas Laboratory 1L-217 instrument.

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